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# Geographical Differences in Human Herpesvirus 8 Seroepidemiology: A Survey of 1,201 Individuals in Asia

**Li-Min Huang,[1]* Shen-Yi Huang ,[2] MaoYuan Chen,[2] MiaoFen Chao,[1] Chun-Yi Lu,[1] Hwei-Fang Tien,[2] Chin-Yun Lee,[1] and Kuan-Teh Jeang[3]**

[1]*Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan*
[2]*Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan*
[3]*Section of Molecular Virology, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Health of Health, Bethesda, Maryland*

Since the discovery of human herpesvirus 8 (HHV8) as a contributory cause of Kaposis sarcoma, the clinical role of this virus has been actively investigated. An understanding of HHV8 seroepidemiology is critical for the study of its pathogenesis within a specific environment. A sero-survey is described in Taiwan of 1,201 individuals ranging in age from under 1 year to over 70. Indirect immunofluorescence assay was used to determine antibody titers against both latent and lytic antigens of HHV8. The results indicate that very few individuals (3–4%) were exposed to HHV8 before 10 years of age. Infection rate peaked (19.2%) between the ages of 21 to 40. Females showed a slightly higher seroprevalence for HHV8 than males, but the difference was not statistically significant. Pregnancy did not correlate with increased HHV8 infection rate nor with augmented HHV8 antibody titers. It is concluded that HHV8 in Taiwan is predominantly an infectious agent for adults. In this geographical locale, HHV8 is similar to herpes simplex virus type 2 in its likely transmission occurring presumptively through sexual routes. However, the study also indicates that a smaller portion of HHV8-transmission could occur through nonsexual contacts. ***J. Med. Virol. 60: 290–293, 2000.*** 

**KEY WORDS:** HHV8; antibody; sexually transmitted disease

## INTRODUCTION

Kaposis sarcoma (KS) is the most common neoplasm in patients with the acquired immunodeficiency syndrome (AIDS). Approximately 15% to 20% of AIDS patients develop KS. Thus, the risk for KS in this population is 20,000-fold higher than that for the general population [Beral, 1991]. Epidemiologic data suggest that AIDS-associated KS (AIDS-KS) has an infectious etiology and that the human immunodeficiency virus (HIV) is not the sole determinant of KS [Beral et al., 1990; Beral, 1991]. Findings supportive of a non-HIV etiology for KS include the following. First, KS occurs at significant rates in selected HIV-negative groups, including immunosuppressed transplant recipients and well-defined African and Mediterranean populations. Second, even among HIV infected individuals the risk for KS varies widely, with high rates observed in HIV-positive homosexual adult men and very low rates among HIV-infected hemophiliacs and children [Blauvelt et al., 1997]. These observations have led to the deduction that a second, sexual transmitted cofactor might be involved in KS etiology or pathogenesis. Using representational difference analysis Chang et al. [1994] discovered novel viral DNA sequences in Kaposi's sarcoma (KS) tissues from AIDS patients. This landmark observation led to the identification of a new gamma-herpes virus, first designated as KS-associated herpesvirus (KSHS) and later renamed human herpesvirus 8 (HHV8) [Ambroziak et al., 1995]. Emerging evidence suggests that this is an entirely unique herpesvirus and contains 170 kb [Renne et al., 1996a].

HHV8 sequences are present in more than 90% of AIDS-KS tissues as well as in the majority of HIV-negative KS [Huang et al., 1995; Moore and Chang 1995; Schalling et al., 1995; Chuck et al., 1996; Su et al., 1996]. HHV8 is also found in Castleman's disease

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*Correspondence to: Dr. Li-Min Huang, Department of Pediatrics, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei, Taiwan. E-mail: lmhuang@ha.mc.ntu.edu.tw

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TABLE I. HHV8 Seropositive Rates in General Population in Taiwan

| Age | <5 Y | | 6–10 Y | | 11–20 Y | | 21–30 Y | | 31–40 Y | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of total cases | 200 | | 100 | | 132 | | 167 | | 120 | |
| Seropositive | 6 (3%) | | 4 (4%) | | 16 (12.1%) | | 26 (15.6%) | | 23 (19.2%) | |
| Range of antibody titers | 10–40 | | 10–40 | | 10–320 | | 10–80 | | 10–320 | |
| GMT | 13.2 | | 14.1 | | 27.7 | | 16 | | 21.9 | |
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| Seropositive (%) | 3.7 | 6.5 | 3.9 | 4.1 | 7.4 | 7.3 | 13.7 | 18.1 | 15.5 | 27.8 |
| Age | 41–50 Y | | 51–60 Y | | 61–70 Y | | >70 Y | | Pregnant mothers | |
| Number of total cases | 115 | | 125 | | 60 | | 74 | | 134 | |
| Seropositive | 9 (7.8%) | | 16 (12.8%) | | 5 (8.3%) | | 10 (13.5%) | | 26 (24.1%) | |
| Range of antibody titers | 10–640 | | 10–640 | | 10–640 | | 10–640 | | 10–640 | |
| GMT[a] | 18.5 | | 24.8 | | 30.3 | | 42.9 | | 17.5 | |
| | Male | Female | Male | Female | Male | Female | Male | Female | | |
| Seropositive (%) | 7.3 | 9.1 | 11.8 | 14.3 | 8.8 | 7.7 | 14.5 | 10.5 | | |

[a]GMT, geometric mean titer.

[Soulier et al., 1995], a rare lymphoproliferative disorder often associated with KS, and in some unusual high-grade body cavity-based lymphomas in HIV-infected patients [Cesarman et al., 1995]. Some studies have suggested that HHV8 is also present in various tissues of healthy individuals, including peripheral blood mononuclear cell [Whitby et al., 1995], prostate tissue and human semen [Monini et al., 1996], and nasal secretions and saliva [Boldogh et al., 1996; Koelle et al., 1997; Levy 1997]. However, in AIDS patients who were HHV8 PCR-positive in peripheral blood mononuclear cells but did not have KS at the time of testing, the risk of subsequent KS development was substantially higher than those in whom HHV8 was not detected [Whitby et al., 1995]. These observations, while not providing direct proof, are largely consistent with a contributory role of HHV8 in KS-development.

Despite intensive investigation, how HHV8 is spread and the magnitude of this spread within defined populations remain incompletely understood. Thus, the seroprevalences of HHV8 in "general" populations range quite differently depending upon geographical locales. Hence, in developed countries, depending on the study, 1%–25% of the general population are HHV8 seropositive [Gao et al., 1996; Simpson et al., 1996; Lennette et al., 1996; Martin et al., 1998] By contrast, the infection rates are much higher in developing countries. HHV8 DNA has been detected in 22.5% of peripheral blood mononuclear cells of blood donors in central Africa [Belec et al., 1998]; a seropositive rate of 50% has been reported for children in Uganda [Mayama et al., 1998]; and a dramatically high seropositivity of 80-100% has been observed in blood samples from Gambia and Ivory Coast [Lennette et al., 1996]. Although interlaboratory and interassay variation may account for some of the differences in HHV8 seroepidemiology [Rickinson et al., 1996; Levy, 1997; Sharp, 1998], it is expected that, like other herpesviruses [Sumaya, 1998], socioeconomic status remain an important determinant of HHV8 infection in different populations.

In contrast to the many studies from the United States, Europe, the Mediterranean, and Africa, there is very little known about HHV8 in Asia. Taiwan is a developing country which has high prevalences of several herpesvirus-associated human diseases and malignancies including Epstein-Barr virus associated nasopharyngeal carcinoma and peripheral T cell lymphoma [Su et al., 1991; Chang et al., 1995]. Herpesvirus infection has also been reported to induce more frequently certain unique manifestations such as hemophagocytosis syndrome in Taiwan [Su et al., 1989; Huang et al., 1990]. In order to compare and contrast with findings from other regions of the globe, a study was undertaken a study to understand better the infection status of HHV8 in Taiwan.

## SUBJECTS AND METHODS

### Subjects

The study consisted of 1,201 serum samples collected between 1994 and 1998 from people living around Taipei City, including students of kindergarten and schools. Most of the adult serum samples were derived from a volunteer-donated blood bank. All subjects were apparently healthy and experienced no acute illness at the time of blood sampling. Verbal consent was obtained from each subject and/or his/her guardian. The study population covered a wide range of ages, from newborn to adults (Table I). Sera were collected by centrifugation of blood at 1500 rpm for 5 minutes and then stored at −20°C until tested.

### Serologic Assay

Indirect immunofluorescence assay (IFA) was used to test for the antibody titers against HHV-8 [Lennette et al., 1996]. The BCBL-1 cells, which are infected latently with HHV 8 but not infected with the Epstein-Barr virus, were used in this assay [Renne et al., 1996b]. Cells were activated by treatment with TPA (20 ng/ml) for 5 days. Thereafter, the cells were fixed onto glass slides by cold acetone and blocked by incubation with PBS containing 5% BSA for 30 min in a humidified chamber. The slide was then overlaid with patient serum diluted 1:10 in blocking solution (5% BSA in PBS) and incubated for 1 hr. Unbound serum was washed away with PBS for 10 min repeated 3 times. The secondary antibody, mouse anti-human $IgG_{123}$, diluted 1:2000 in blocking solution was then added and incubated for 1 hr. Washing with PBS for 3 times was carried out again followed by the addition of FITC-conjugated goat anti-mouse IgG diluted 1:4000 in blocking solution and incubated for another hour. After the final wash, the slide was observed under immunofluorescence microscope with mounting medium (PBS:glycerol = 1:9). Titer of a specific serum was defined as the reciprocal of highest dilution, which gave positive fluorescence. The reciprocal of the highest dilution that provided positive fluorescence in at least 5% of cells was defined as the antibody titer. A titer of 10 or more was considered positive. Several serum samples derived from patients with Kaposi's sarcoma were tested positive and served as positive controls for the serologic assay. The assay was read by two persons, one of whom was blind to the sample identity. All the fluorescence readings in this study were in good agreement between the two examiners. There was no difference of more than 2 fold in final antibody titer assignment.

### Statistical Analysis

Groups were compared using Student's t test for two means, two-tailed. Antibody titers were log transformed first for statistical comparison. Seropositive rates were compared between groups using chi-square test with Yates' correction. A *P* value less than 0.05 was considered significant.

## RESULTS

1,201 serum samples were assayed for IgG anti-HHV8 antibody. The age spectrum spanned newborn to over 70 years in age; the study also contained a group of 108 pregnant women (Table I). Males (653) slightly outnumbered females (548) in this study because more male blood donors were included than female ones.

HHV8-seropositives in individuals under 10 years of age were low (3%–4%); this number started to rise in subjects between 11 to 20 years of age (12.1%). The rate peaked in the age group between 31 and 40 years (19.2%). Thereafter, rates declined to around 10%. Table I shows the seropositive rates of males and females. Although females between the age 21 years and 60 years had higher seropositive rates than counterpart males, no statistically significant differences were readily appreciated between sexes in each of the different age stratum. The seropositive rate (24.1%) and geometric mean titers (GMT = 17.5) of pregnant women were similar to the rate (21.3%) and GMT (25.9) of women aged from 21 to 40 years. Geometric mean titers rose with age, the highest titer was observed in subjects over 71 years of age.

## DISCUSSION

Understanding the seroepidemiology of viral infections is critical to elucidating viral spread and to predicting the age when primary viral infection is likely to occur. Based on experiences with other viruses, it is well understood that transmission patterns in one geographical and socioeconomic cannot be easily predicted from data from divergent locales. While much has been reported on HHV8 seroprevalence elsewhere, there is a dearth of information for this virus in Asia. A relatively large population of otherwise healthy individuals in Taiwan was assessed for HHV8 serostatus. The study indicates that similar to findings from the United States and in contrast to findings from Africa, HHV8 infection is rare (3%–4%) before 10 years of age. Most of the infection in Taiwan was noted to occur between 10 and 40 years of age.

A virological issue regarding HHV8 is how does the virus spread? HHV8 and/or its genome have been detected in semen, blood, and nasopharyngeal aspirates [Whitby et al., 1995; Boldogh et al., 1996; Monini et al., 1996; Koelle et al., 1997; Levy, 1997]. In principle, therefore, HHV8 transmission can occur through exchanges of sexual (semen) and non-sexual (saliva or nasopharyngeal secretion) bodily fluids. One way to view HHV8 transmission is to compare this virus to other herpesviruses with well-delineated modes of transmission. Thus amongst the herpes viruses, HSV2 is a prototypic for sexual transmission [Kohl, 1998], while EBV is understood to spread by contact involving exchanges of nasopharyngeal secretion and saliva [Sumaya, 1998]. For the situation in the Taiwan population, we found that HHV8 infection was similar to that of HSV-2 rather than EBV in view of the rare infection before puberty (3%–4%, see Table I) and highest infection rate in young adults between 20 and 40 years of age (15%–19%, see Table I). Our observation is most compatible with a sexual route for transmission of HHV8 and agrees with comparable studies in two other populations [Levy, 1997; Sosa et al., 1998].

Recently, many studies used immunofluorescence assay (IFA) to determine the HHV8 antibody. However, it should be noted that there remains many different ways to perform IFAs for HHV8. Thus, differences reside in the use of cell lines for antigen sources and the means to activate HHV8 inside cells. In this study the assay developed by Lennette et al. [1996] was used since it appears to be one of the more sensitive assays in that it could detect reliably HHV8 antibody in almost all serum samples of patients with Kaposi's sarcoma [Rickinson, 1996]. Within the limits of this detection technique, it is concluded that HHV8 infection in Taiwan spreads through a mode more similar to that in the United States than that in Africa [Gao et al., 1996; Lennette et al., 1996 Simpson et al., 1996;; Blauvelt et al., 1997; Martin et al., 1998; Mayama et al., 1998].

An unanswered question is why would the route(s) of transmission be apparently different between Africa and Taiwan/United States. Perhaps this apparent difference is only a reflection of the much larger viral burden in the former compared to the latter settings. In settings where viral burdens are very high, the less efficient route of transmission through non-sexual bodily fluids occurs successfully at an earlier age. Once this occurred it then masks the future potential for sexual transmission. Indeed the finding of 3%–4% HHV8 seroprevalance in individuals under the age of 10 years in Taiwan would be in agreement with such an interpretation.

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